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Applicant: Bruck, et al. November 7, 2001
Serial No.: 09/509,239 Group Art Unit No.: 1648
Filed: March 23, 2000 Examiner: U. Winkler
For: "Fusion Proteins Comprising HIV-1 Tat and/or Nef Proteins"

Assistant Commissioner for Patents
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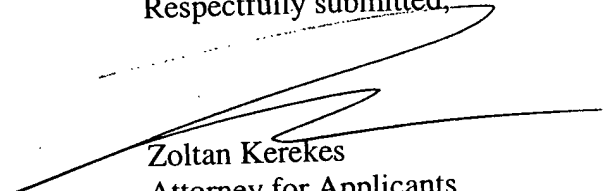
TRANSMITTAL OF FORMAL DRAWINGS

In response to the Notice of Informal Drawings mailed on October 19, 2001 attached
please find:

- (a) the formal drawings for this application with each sheet indicating the Serial
Number and the Group Art Unit. Number of Sheets: 18
- (b) a copy of the Notice of Informal Drawings.

Respectfully submitted,

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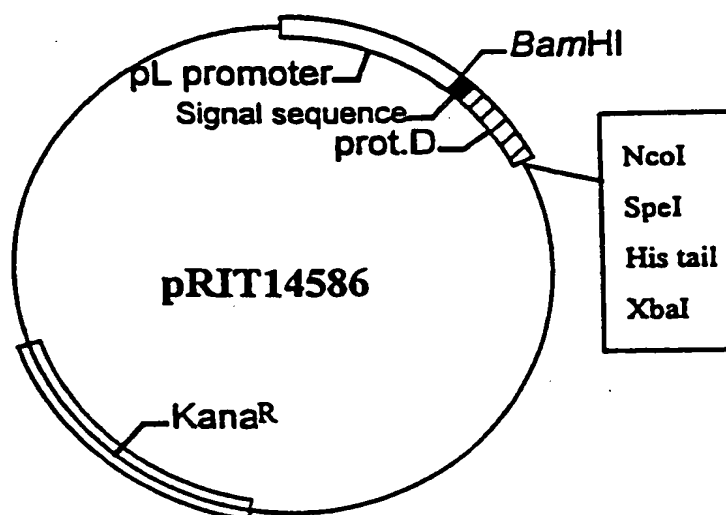

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FIG. 1A Map of plasmid pRIT14586



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FIG. 1B Coding sequence of the first 127 amino acids of protein D and multiple cloning site. The signal sequence is underlined.

BamHI
ATG GAT CCA AAA ACT TTA GCC CTT TCT TTA TTA GCA GCT GGC GTA CTA GCA GGT TGT AGC AGC
 Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu Ala Gly Cys Ser Ser
 CAT TCA TCA AAT ATG GCG AAT ACC CAA ATG AAA TCA GAC AAA ATC ATT ATT GCT CAC CGT GGT
 His Ser Ser Asn Met Ala Asn Thr Gln Met Lys Ser Asp Lys Ile Ile Ile Ala His Arg Gly
 GCT AGC GGT TAT TTA CCA GAG CAT ACG TTA GAA TCT AAA GCA CTT GCT TTT GCA CAA CAG GCT
 Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Gln Ala
 GAT TAT TTA GAG CAA GAT TTA GCA ATG ACT AAG GAT GGT CGT TTA GTG GTT ATT CAC GAT CAC
 Asp Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val Ile His Asp His
 TTT TTA GAT GGC TTG ACT GAT GTT GCG AAA AAA TTC CCA CAT CGT CAT CGT AAA GAT GGC CGT
 Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe Pro His Arg His Arg Lys Asp Gly Arg
 TAC TAT GTC ATC GAC TTT ACC TTA AAA GAA ATT GAA AGT TTA GAA ATG ACA GAA AAC TTT GAA
 Tyr Tyr Val Ile Asp Phe Thr Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu
NcoI SpeI XbaI
ACC ATG GCC ACG TGT GAT CAG AGC TCA ACT AGT GGA CAC CAT CAC CAT CAC CAT TAA TCT AGA
 Thr Met Ala Thr Cys Asp Gln Ser Ser Thr Ser Gly His His His His His His *

The amino acid sequence of Figure 1 relates to Seq. ID no. 7 and the nucleic acid sequence of Figure 1 relates to Seq. ID no. 6.

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FIG. 2A

The DNA and amino acid sequences of Nef-His; Tat-His; Nef-Tat-His fusion and mutated Tat is illustrated.

Pichia-expressed constructs (plain constructs)

⇒ Nef - HIS

DNA sequence (Seq. ID. No. 8)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 9)

MGGKWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWICYKLVPVEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ Tat - HIS

DNA sequence (Seq. ID. No. 10)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAA

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FIG. 2B

TCCCGAGGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence (Seq. ID. No. 11)

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVSLSKQPTSQSRGDPTGPKETSGHHHHHH.

⇒ Nef - Tat - HIS

DNA sequence (Seq. ID. No. 12)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAG
CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAAGTCTGCT
TGTACCAATTGCTATTGTAAAAGTGTTGCTTTTATTGCCAAGTTTGTTCATAACA
AAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCT
CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGA
GGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 13)

..
MGGKWSKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWICYKLVPVEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTA
CTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSR
GDPTGPKETSGHHHHHH.

E.coli-expressed c nstructs (fusion c nstructs)

⇒ LipoD-Nef-HIS

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FIG. 2C

DNA sequence (Seq. ID. No. 14)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.
The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
AGCAGCCATTTCATCAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
GCTTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
CGTTTAGTGGTTATTCACGATCACTTTTATAGATGGCTTGACTGATGTTGCGAAAAA
TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
GAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGCAAGTGGTCA
AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATT
CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTT
GGATGGTGTCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGA
GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence of the processed lipidated ProtD-Nef-HIS protein (Seq. ID. No. 15)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
GRLVVIHDFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMENFETMGGKW
SKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQG
YFPDWQNYTPGPGVRYPLTFGWICYKLVPEPDKVEEANKGENTSLHPVSLHGMDDP
EREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ LipoD-Nef-Tat-HIS

DNA sequence (Seq. ID. No. 16)

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FIG. 2D

Nucleotides corresponding to the Prot-D Fusion Partner are in bold.
 The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino-terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGTTGT
AGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
GCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
CGTTTAGTGTTATTCACGATCACTTTT**TAGATGGCTTGACTGATGTTGCGAAAAA**
TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
GAAATTCAAAGTTTAGAAATGACAGAAACTTTGAAACCATGGGTGGCAAGTGGTCA
 AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
 GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
 AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
 GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATT
 CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
 TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGT**CAGATATCCACTGACCTTT**
GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTCATCACGTGGCCCGA
GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTA
GAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACTGCTTGTACCAATTGCTATTGT
AAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTTCATAACAAAAGCCTTAGGCATCTCC
TATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCAT
CAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCG
AAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence of the processed lipidated ProtD-NEF-TAT-HIS protein (Seq. ID. No. 17)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFAQQADYLEQDLAMTKD
GRLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEM**TENFETMGGKW**
SKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
EEEVGFVPTPQVPLRPMTYKAAVDLSHFLKEKGGL**EGLIHSQRRQDILD**LWIYHTQ**G**
YFPDWQNYTPGPGVRYPLTFGWCYKLVPVE**PDKVEEANKGENTSLLHPVSLHGMDDP**
EREVLEWRFDSRLAFHHVARELHPEYFK**NC**SEPVDPRLEPWKH**PGSQPKTACTN**CY
CKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTG
PKETSGHHHHHH.

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FIG. 2E

⇒ ProtD-Nef-HIS

DNA sequence (Seq. ID. No. 18)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGTTATTCACGATCACTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAA
GGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
CATCACCATTAA

Protein sequence (Seq. ID. No. 19)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIILAHRGASGYLPEHTLESKALAFQAQADYL
EQDLAMTKDGRLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLK
EIQSLEMENFETMGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRD
EKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSH
FLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGW
CYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFH
HVARELHPEYFKNCTSGHHHHHH.

⇒ ProtD-Nef-Tat-HIS

DNA sequence (Seq. ID. No. 20)

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FIG. 2F

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAA
GGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAGTGCCTAGTGAGCCAGTAGAT
CCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAAT
TGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTA
GGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGT
CAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
ACAGGCCCGAAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 21)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMT
KDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGG
KWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEA
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWLYHT
QGYFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLHGMD
DPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLPEWKHPGSQPKTACTN
CYCKKCCFHCQVCFITKALGISYGRKKRRQRRPPQGSQTHQVSLSKQPTSQSRGDP
TGPKETSGHHHHHH.

⇒ Tat-MUTANT-HIS

DNA sequence (Seq. ID. No. 22)

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FIG. 2G

```

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATC 40
CAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTG 80
TAAAAAGTGTGTGCTTTCATTGCCAAGTTTGTTCATAACA 120
GCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGAC 160
AGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGT 200
TTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAG 240
CCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATC 280
ACCATTA 288

```

Protein sequence(Seq. ID. No. 23)

Mutated amino-acids in Tat sequences are in bold.

```

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFIT 40
AALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGE 80
PTGPKETSGHHHHHHH. 95

```

⇒Nef-Tat-Mutant-HIS

DNA sequence(Seq. ID. No. 24)

```

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGC 40
CTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC 80
AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACAT 120
GGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTG 160
CTTGTCCTGGCTAGAAGCACAAAGAGGAGGAGGAGGTGGG 200
TTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACT 240
TACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAAGAAA 280
AGGGGGGACTGGAAGGGCTAATCACTCCCAACGAAGACA 320
AGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC 360
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCA 400
GATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACC 440
AGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAG 480
AACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGG 520
ATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAG 560
CCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCG 600
GAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTA 640
GACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAC 680
TGCTTGTAACCAATTGCTATTGTAAAAAGTGTGCTTTTCA 720
TGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCT 760
ATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCA 800
AGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC 840
ACCTCCCAATCCAAAGGGGAGCCGACAGGCCCGAAGGAAA 880
CTAGTGGCCACCATCACCATCACCATTAA 909

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FIG. 2H

Protein sequence (Seq. ID. No. 25)

Mutated amino-acids in Tat sequence are in bold.

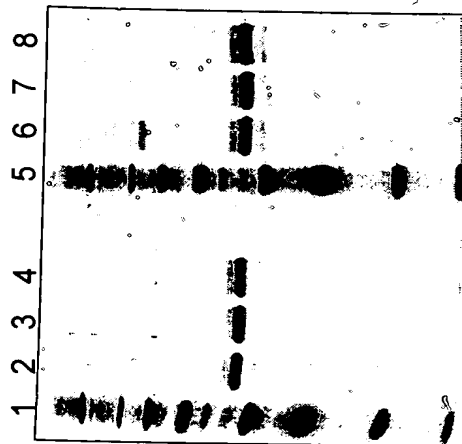
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GAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMT  80
YKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGY 120
FPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGE 160
NTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHP 200
EYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFH 240
CQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQP 280
TSQSKGEPTGPKETSGHHHHHH.                      302

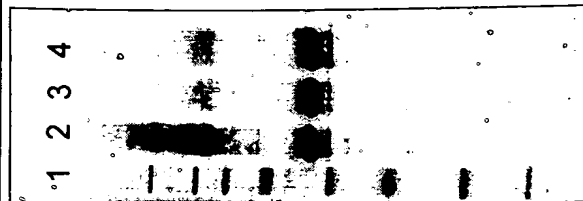
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FIG. 4 SDS-PAGE: Nef-Tat-his fusion protein

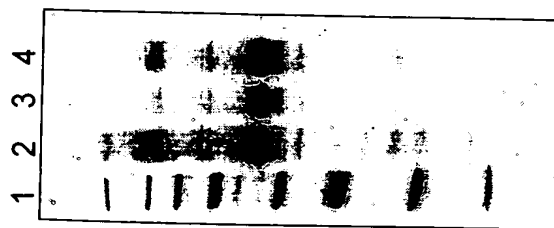
- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa
- 2: TNH/23 SP eluate (250 ng)
- 3: TNH/23 Purified bulk (250 ng)
- 4: TNH/22 Purified bulk (250 ng)
- 5: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa
- 6: TNH/23 SP eluate (400 ng)
- 7: TNH/23 Purified bulk (400 ng)
- 8: TNH/22 Purified bulk (400 ng)



Daiichi Silver Staining



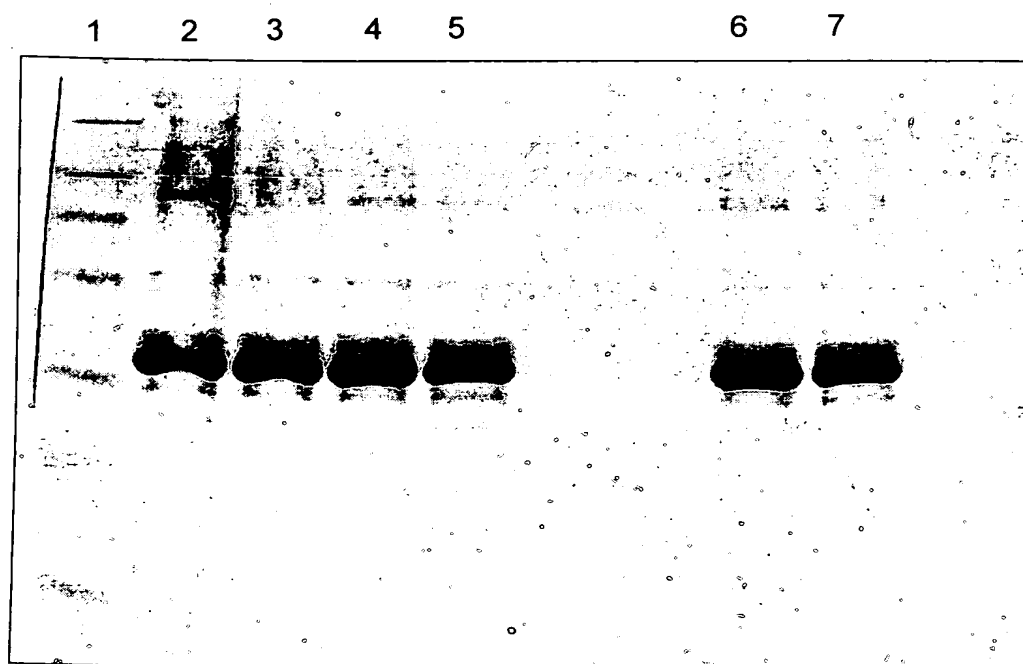
Blot α Nef-Tat (LAS 97340)



Blot Tat2

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FIG. 5 SDS-PAGE: Nef-Tat-his fusion protein



Coomassie blue G250

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa)
- 2: TNH/23 SP eluate (4 μ g)
- 3: TNH/23 Superdex200 eluate (4 μ g)
- 4: TNH/23 Purified bulk (4 μ g)
- 5: TNH/22 Purified bulk (4 μ g)
- 6: TNH/23 Purified bulk (4 μ g) / non reducing conditions
- 7: TNH/22 Purified bulk (4 μ g) / non reducing conditions

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FIG. 6A Tat-specific antibody titers and isotypes

group	immunization	midpoint titers					ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b		
1	oxydized Tat	353557	135538	98771	98763	1,372	
2	reduced Tat	252275	72087	76273	72014	0,945	
3	oxydized Nef-Tat	246466	179616	60835	53563	2,953	
4	reduced Nef-Tat	91726	73767	30948	20679	2,384	
5	adjuvant only	<4000	<4000	<4000	<4000		

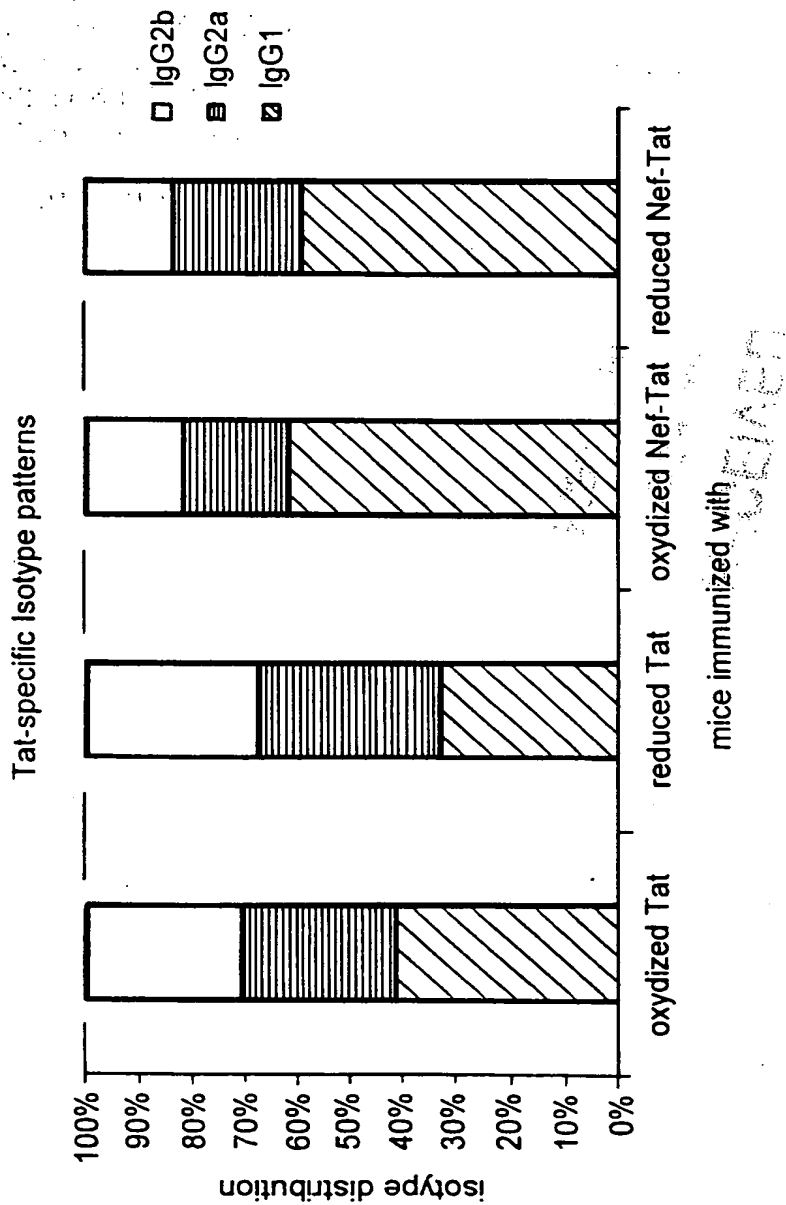


FIG. 6B Tat-specific antibody titers and isotypes

group	immunization	midpoint titers				ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b	
1	reduced Tat	212799	123242	62697	55763	1,966
2	reduced Nef-Tat	75676	84046	18449	11692	4,556
3	adjuvant only	<4000	<4000	<4000	<4000	

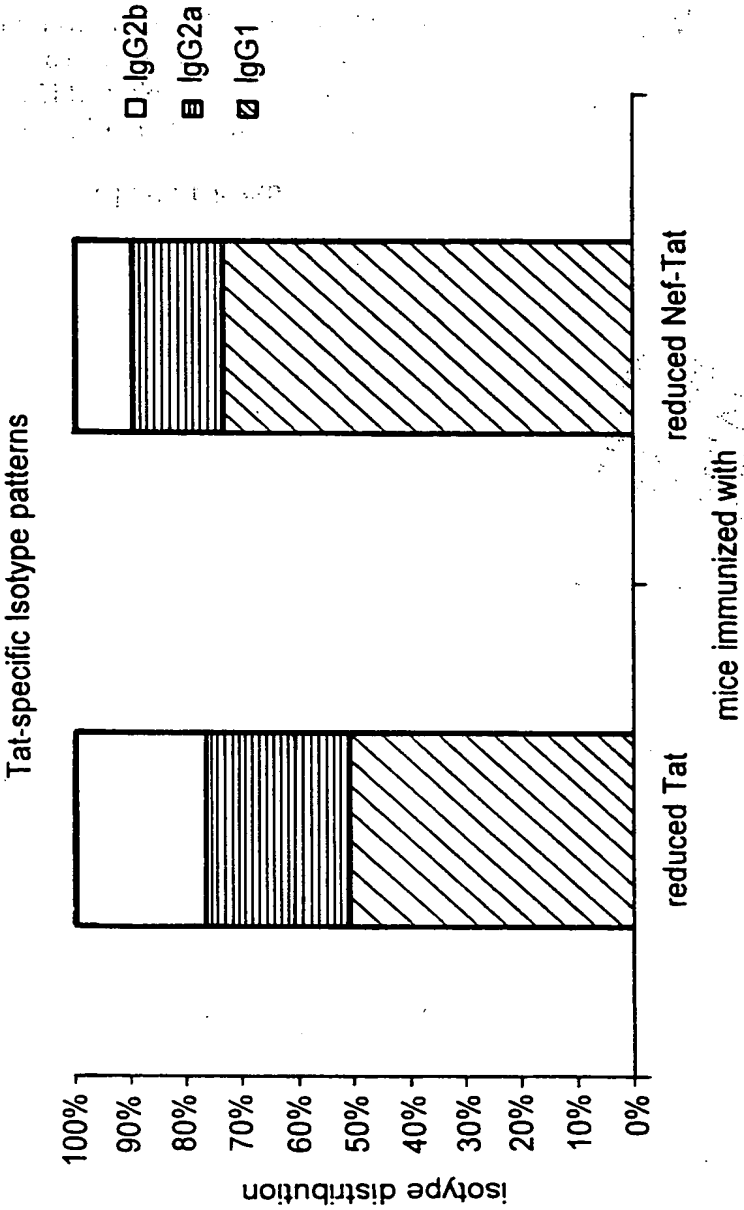


FIG. 7

Antigen-specific lymphoproliferative response of pooled lymph node cells

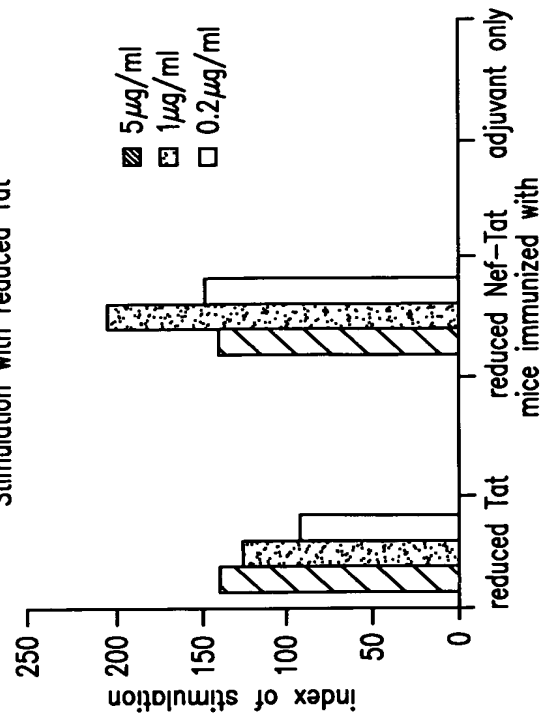
(3H) Thymidine incorporation in cpm

Data expressed as stimulation index

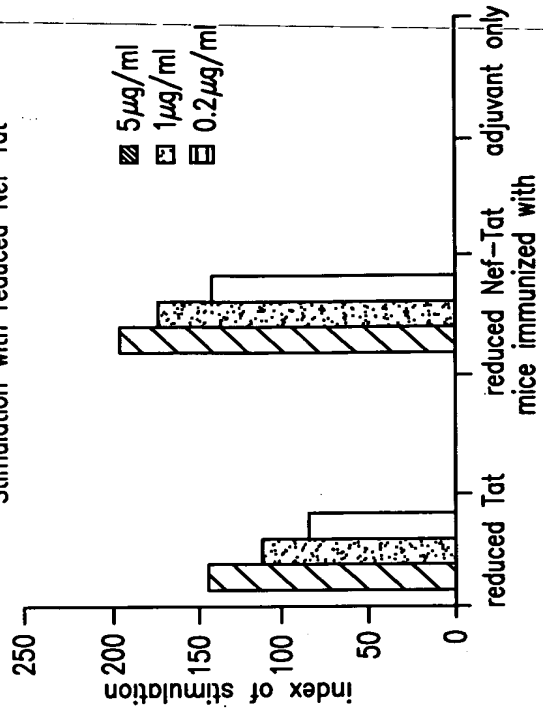
	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat 5 μ g/ml 1 μ g/ml 0.2 μ g/ml	41967 37609 27640	18511 32346 23408	789 415 397
reduced Nef-Tat 5 μ g/ml 1 μ g/ml 0.2 μ g/ml	43882 33865 25079	31694 28094 22891	483 245 383
medium	300	161	571

	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat 5 μ g/ml 1 μ g/ml 0.2 μ g/ml	140 125 92	115 201 145	1 1 1
reduced Nef-Tat 5 μ g/ml 1 μ g/ml 0.2 μ g/ml	146 113 84	197 174 142	1 0 1
medium	1	1	1

Stimulation with reduced Tat



Stimulation with reduced Nef-Tat



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FIG. 8A Cell binding assay

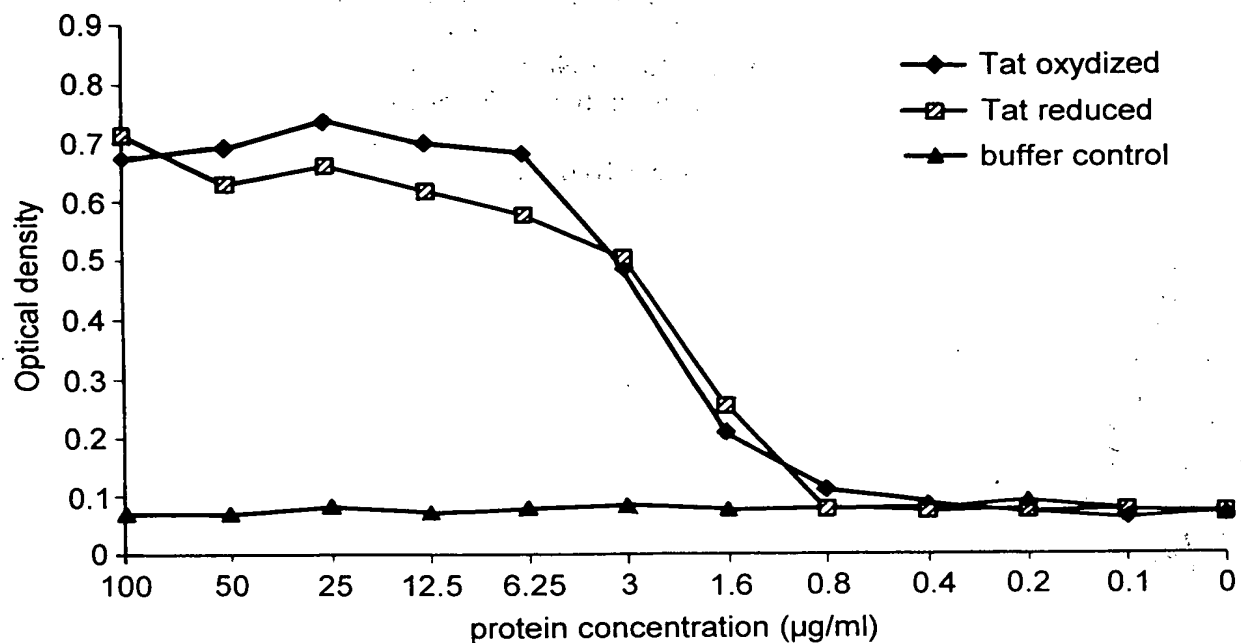
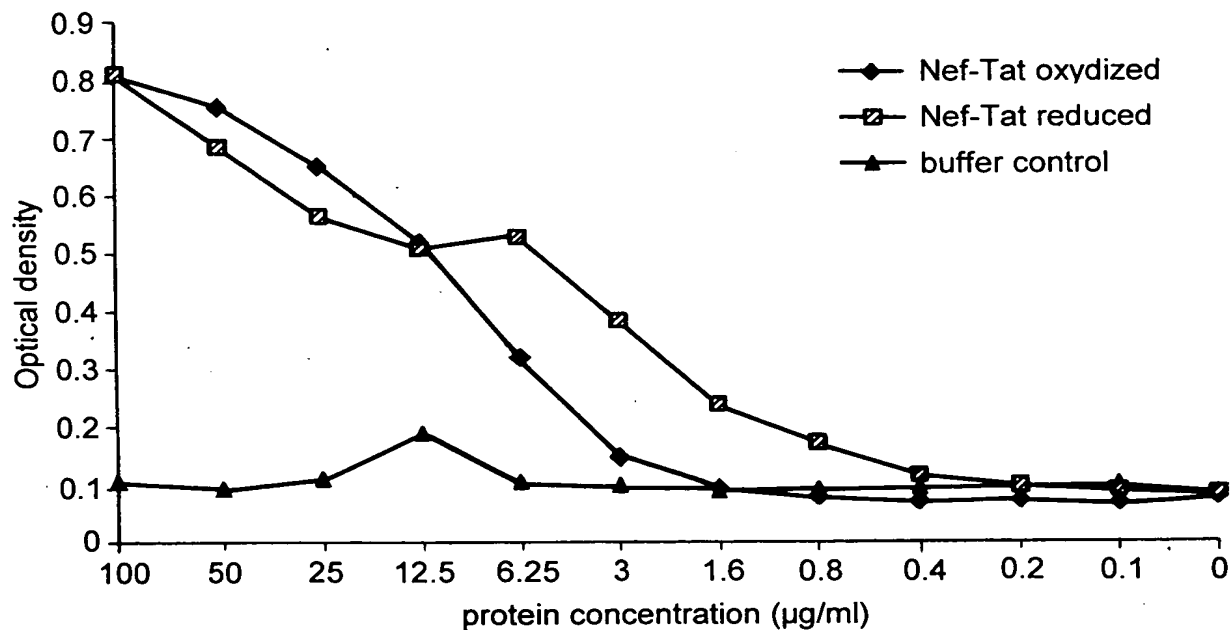


FIG. 8B



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FIG. 9A Inhibition of cell growth

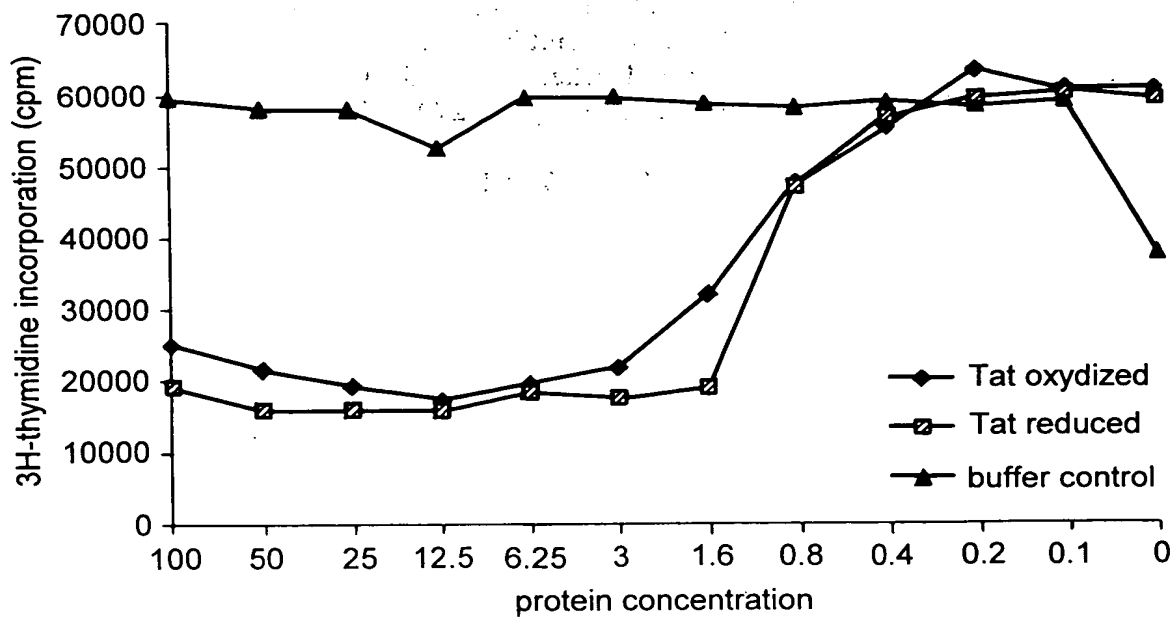


FIG. 9B

